REMARKS/ARGUMENTS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

New claims 62-68 have been added. These new claims further define the pre-determined metabolic signal (e.g., ion concentration, pH change, or change in gas concentration). New claims 62 and 63 find basis in the disclosure at the bottom of page 5; new claim 64 finds basis in the disclosure at the bottom of page 17; new claim 65 finds basis in the disclosure at the bottom of page 35; new claim 66 finds basis in the disclosure at the bottom of page 35; new claim 67 finds basis in the disclosure at the bottom of page 1; and new claim 68 finds basis in the disclosure at the bottom of page 17.

Claims 42-45, 51, 52, 54, 55, 58 and 61 stand rejected under 35 USC 103 as allegedly being obvious over Meers et al (USP 6,339,069) in view of Parente et al. The rejection is traversed.

At the outset, Applicants note that the application that issued as the cited Meers et al patent was filed on June 29, 1999, that is, after the filing date of the PCT application from which this case derives (October 14, 1998). Accordingly, the Examiner is requested to withdraw the rejection or identify in which of the earlier filed Meers et al applications subject matter viewed as relevant by the Examiner is found.

The Examiner's attention is directed to the fact that in an earlier Office Action (dated November 20, 2003), a related Meers et al patent, USP 6,087,325 (which issued from an application filed October 15, 1997) was cited. Applicants successfully addressed that rejection on the basis that the '325 patent does not disclose a cytolytic peptide that *interacts* with the lipid

layer. Nevertheless, for the purposes of this response, Applicants explain below why the instant claims would not have been obvious over the art upon which the Examiner relies.

Claim 42 relates to a highly sensitive method for *detecting* target cells in a sample. The method is limited to adding to the sample highly stable liposome particles having a cytolytic peptide, which are targeted to the target cells, which release a pre-determined metabolic signal. In response to the metabolic signal released by the target cells, the cytolytic peptide, already assembled into the hydrocarbon lipid phase of the bilayer membrane, *interacts* with it to modulate the permeability of the particles, whereupon a species is activated, to thereby allow for the detection of the target cells.

Applicants submit that the cited art does not disclose a method of *detecting* target cells in a sample. In addition, art does not the use of particles involve as defined by claim 42 . Hence, as the Examiner acknowledges, claim 42 is novel.

All remaining claims are novel by virtue of their dependency on claim 42. In particular, claims 62 to 68 are novel over Meers et al (US 6,339,069), which only discloses the action of a peptidase to cleave the peptide from the aqueous periphery of the lipid bilayer. In contrast, new claims 62-68 recite a range of different metabolic signals, which can be used in the detection method, other than a peptidase enzyme as in Meers et al.

As pointed out above, Meers et al does not disclose a method of detecting target cells. Meers et al discloses the use of a non-cytolytic, stabilizing peptide that is covalently attached to the hydrophilic lipid head groups of the lipid layer of the liposome for treating diseases. The peptide acts as a blocking group to stabilize intrinsically *unstable* liposomes. Upon peptidase cleavage of the peptide, the bilayer structure of the membrane destablizes, thereby causing the unstable Meers et al liposome to fall apart by virtue of disruption of the aqueous periphery as

opposed to the hydrocarbon phase. Hence, the cytolytic peptides used in the present invention are fundamentally different from the peptides disclosed in Meers et al.

The Examiner acknowledges that Meers et al does not disclose the use of a *cytolytic* peptide as required by claim 42. However, the Examiner alleges that it would have been obvious to combine Meers et al with Parente et al, which discloses use of the peptide, GALA (i.e. a cytolytic peptide). Applicants strongly disagree. First, neither Meers et al nor Parente et al discloses a method of detecting target cells in a mixed sample. Hence, even if these documents were to have been combined (which they would not have been), they would still not have suggested the method as claimed in claim 42, which is clearly limited to a method of *detection*.

Furthermore, the methods disclosed in Meers et al and Parente et al are intrinsically incompatible and, hence, one skilled in the art would not have combined their teachings. Meers et al discloses a method of treating diseases, whereas Parente et al is a purely academic paper discussing GALA. Hence, the skilled technician would have found no motivation to combine the teaching of these two documents. Thus, the invention could not have been obvious.

In addition to the above, Applicants point out that, for the reasons that follow, nothing in the cited art would have suggested that the claimed invention would work:

a) Parente et al clearly discloses that such peptides only associate with lipid membranes when they are in the lytic, pH-activated state. The skilled technician would, therefore, have expected that if the peptides were associated with liposomes in this lytic, pH-activated state, that they would lyse the liposomes before any biological pH activity had the opportunity to activate the peptides. Hence, the artisan would never have attempted to pre-associate such lytic peptides with liposomes, as he/she would have expected that

the liposomes would be instantly lysed. Accordingly, claim 42 would not have been obvious.

b) In any typical biological application, such as carrying out the method for detecting target cells in a sample of food or tissue, this problem is considerably worse and, again, would, therefore, never have been attempted by one skilled in the art. The artisan would have known that there would be large quantities of cells in the sample, and would have expected that the cell membranes in the sample would "mop-up" any free lytically-active peptide and prevent it acting upon the liposomes. Thus, in a sample with a small number of target cells and many orders of magnitude more non-target cells present, the skilled technician would have envisaged that any active peptide would be very unlikely to locate the liposomes at sufficient levels to lyse the liposomes. Hence, the artisan would not have combined Meers et al with Parente et al to arrive at claim 42.

c) In natural biological action upon membranes, cytolytic peptides are produced by cells and released at the necessary high concentrations at the site of action in order to affect the level of localized control necessary. Such anti-microbial cytolytic peptides are well known and appear widely in nature from insects to humans. Such cytolytic peptides are activated and released by cells (e.g., of the immune system) proximal to their site of action. In this way, naturally, the peptides are targeted and do not significantly lyse cells elsewhere. If the peptides were not associated with the liposomes and supplied at sufficient concentrations in cell detection assay solutions to lyse liposomes also present, for the aforementioned reasons, it was also well known that such peptide concentrations would lyse and kill the cells as they do naturally. Therefore, because cells are killed by the use of such peptides, one skilled in the art would not have even considered using the

Parente et al peptides to detect the activity of live cells, as they would prevent any change in pH or other metabolic activities from occurring because the cells would have been lysed and killed by the peptides. Hence, the artisan would not have combined Meers et al with Parente et al, and thus claim 42 would not have been.

d) It is also well-known that even early lysis (e.g., loss of potassium ions from the cytoplasm as an early sign of membrane damage) results in cell metabolism being switched off quickly. Hence, it would have been considered pointless by one skilled in the art to attempt to use lytic peptides disclosed in Parente et al in a detection method in the presence of actively growing cells. Thus, the artisan would not have combined Meers et al with Parente et al.

e) The pH necessary to activate the peptides disclosed in Parente et al is relatively acidic (pH 5 – pH 5.5) in which most target cells of interest would be killed. Hence, any such attempt would not have been expected to work and would, therefore, have been considered useless by one skilled in the art. Hence, the artisan would not have combined Meers et al with Parente et al.

f) Those skilled in the art of lytic peptides would not have considered the combination of Meers et al and Parente et al to provide a workable approach, which, in any case, would not have been relevant to this invention. Nonetheless, for completeness, it is noted that it was known that the attachment of hydrophilic groups such as those of the peptides disclosed in Meers et al would not prevent or control the activity of the peptide. For example, if such a peptide sequence were incorporated within the lytic peptide structure (in the middle of the lytic sequence), where it could block the activity of the peptide, then upon treatment with a protease, which cleaves the Meers et al peptide, activity of the lytic

peptide would be permanently destroyed. The lytic peptide would, therefore, break-up into small oligopeptide fragments that are far too small to be lytic, and so would not be able to modulate the permeability of the particles, as required in claim 42. The alternative of incorporating a Meers et al-type peptide at the terminus of the peptide, such that proteolytic cleavage of the Meers et al-type peptide would leave the lytic peptide sequence intact, would also not have been expected to work. This is because the other end of the peptide (i.e., the lytic peptide sequence) would remain lytic in the presence or absence of the Meers et al-type sequence such that cleavage of the Meers et al type peptide would not activate the lytic portion of the peptide. For example, it was well-known in the art that such lytic peptides could be covalently tethered at their termini to large objects such as plastic spheres and surfaces, when they still remain lytically active. The attachment of a very much smaller Meers et al-type peptide to a Parente et al type peptide would have been expected to have no effect. Hence, combining Meers et al and Parente et al peptides would not have been considered by those skilled in the art, and, in any case, a Meers et al-type peptide or its mechanism of action is not used and could not be used in the context of the present invention.

In conclusion, Applicants submit that one skilled in the art would not have considered using the peptides disclosed in Parente et al with the liposomes disclosed in Meers et al for the purposes of the claimed invention because it would have been expected that the peptides (disclosed in Parente et al) would lyse the liposomes well before any biological activation (e.g., pH change) could occur, and would, therefore, disrupt the liposome before it had itself an opportunity to be activated. Furthermore, the conditions of activation (e.g., acidic pH) would not

be achieved because cells would die or stop metabolizing before they had had a chance to be achieved.

Applicants accidentally combined three features, which unexpectedly worked, to produce a non-obvious invention that is believed to be the most sensitive cell detection method known. The method is many orders of magnitude more sensitive than could have been expected by one skilled in the art, and exhibits the advantages discussed on p.21 of the specification.

Applicants combined:-

a) Pre-associating with liposomes a peptide in a non-lytic form. Hence, there is no significant free peptide available to kill the target cells even when activated because it is associated with liposomes and, therefore, not free to interact with cells;

b) In so doing, it also turned out unexpectedly that, in the example of pH activation (for example, as claimed in claim 64), the pH activation was shifted markedly to be within a typical physiological range of just less than neutral (i.e., pH 6.5) rather acidic (i.e., pH 5) as in the cited art; and

c) Targeting the peptide-associated liposomes specifically to the target cells resulted, unexpectedly, in the ability to detect localized changes at the cell surface for very low cell numbers that could not possibly change the bulk concentration in the medium to a sufficient extent to activate the peptide.

In accordance with the invention, fewer than 10 bacterial target cells can be detected by producing a color change that is visible to the eye. This was totally unexpected because it is well known that at least 100,000 (and usually 10,000,000 cells) per ml are necessary to produce a pH change in growth media sufficient to activate such peptides. This is far away from any expectation of detecting <10 cells, which is actually achievable. Protons are well known to be

highly mobile, such that even weakly buffered media designed to maximize the low levels of protons released from small numbers of cells would become rapidly buffered (mopped-up) by the bulk medium. Therefore, it would have been totally unexpected to those skilled in the art that the protons produced locally from each of 10 cells in a comparatively large bulk of medium (even in a small volume) would be selectively sensed by the peptide embedded in the liposomes close to the cells.

Furthermore, in the art of immunoassay, where particles such as liposomes are targeted to cells captured and concentrated on a surface, it also takes at least 100,000 (and usually 10,000,000) cells to bind sufficient particles to produce sufficient signal (e.g., color) to be detected by a spectrophotometric reader (let alone by eye). It would, therefore, have been totally unexpected that in targeting these peptide-liposomes that as few as 10 cells would produce an eye-visible color change.

The accidental use in this invention of binding agents that produced binding to cells of more liposomes in a localized manner not obscured by binding to a surfaces, surprisingly induces sufficient liposomes in the vicinity of the target cells to produce a visible color change at many orders of magnitude less cells than would have been expected.

In addition, claims 62 to 68 would not have been over Meers et al, which only discloses the action of a peptidase to cleave the peptide from the aqueous periphery of the lipid membrane bilayer. In contrast, the claimed invention takes advantage of a range of different metabolic signals other than a peptidase as in Meers et al. As mentioned above, one skilled in the art would never have combined Meers et al and Parente et al. Furthermore, Parente et al only discloses the increase of permeability of the particle when the pH is reduced to 5.0, which is relatively acidic. New claims 65 and 66 are limited to the feature that the metabolic signal is a change in pH to

above 6, and 7, respectively. An artisan can appreciate that it is a major advantage to be able to use the particles in the claimed method at more physiologically acceptable pH's as opposed to the acidic conditions disclosed in Meers et al.

In view of the above, reconsideration is requested.

Claims 46-50 stand rejected under 35 USC 103 as allegedly being obvious over Meers et al in view of Parente et al and further in view of Li et al. The rejection is traversed.

The fundamental failings of Meers et al, taken alone or in combination with Parente et al, are detailed above. Nothing in any teachings of Li et al regarding the use of binding agents would have cured those deficiencies. Accordingly, reconsideration is requested.

Claims 55-57 stand rejected under 35 USC 103 as allegedly being obvious over Meers et al in view of Parente et al and further in view of Levinson et al. The rejection is traversed.

The distinctions between the present invention and the combination of Meers et al and Parente et al are discussed above. The addition of Levinson et al's teachings relating to delivery would not have brought one skilled in the art any close to the present invention. Accordingly, reconsideration is requested.

Claim 59 stands rejected as obvious over Meers et al in view of Parente et al and Robinson et al. Claim 60 stands rejected as obvious over Meers et al in view of Parente et al and Blondin et al. These rejections are also traversed.

Any teaching in Robinson et al relating to the analysis of food stuffs and any teaching in Blondin et al relating to detection of toxins in water samples would not have cured the failings of Meers et al and Parente et al (discussed in detail above) and thus would not have rendered obvious the subject matter of claims 59 and 60, respectively. Reconsideration is requested.

Claims 42, 53-55, 58 and 61 stand rejected under 35 USC 103 as allegedly being obvious over Meers et al in view of Rizzo et al. Claims 46-50 stand rejected over this same combination taken further in view of Li et al. Claims 55-57 stand rejected as obvious over Meers et al in view of Rizzo et al and Levinson et al. Claims 59 and 60 stand rejected as obvious over Meers et al and Rizzo et al in view of Robinson et al and Blondin et al, respectfully. The rejections are traversed for the reasons that follow.

All of the rejections are based on the combination of Meers et al and Rizzo et al. The differences between the claimed invention and Meers et al are detailed above. Conspicuous by its absence is any comment from the Examiner as to where in either Rizzo et al or Meers et al motivation can be found to combine these two disclosures. Applicants submit that in fact no such motivation exists and that even if the documents had been combined (which they would not have been), that combination would not have rendered the invention obvious.

Given the foregoing, it will be clear that the rejections based on the combination of Meers et al and Rizzo et al with any of Li et al, Levinson et al, Robinson et al and Blondin et al are not well founded.

The Examiner is requested to provide proper support for these rejections or withdraw same.

CLARKE et al
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This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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